

cycle\_a theme predicted based on rigid-body movements of each TMD-NBD complex for the popular alternating access model proposed for ABC transporters at large. Here, we report a CFTR mutant (R352C) that exhibits two distinguishable single-channel conductance levels (O1 and O2). The transition of these two open states follows a preferred order (C-O1-O2-C), indicating an input of free energy that drives the predominant O1-O2 transition over the opposite O2-O1 transition. This idea is further supported by the observation that only C-O1-C was seen in the presence of ATP when introducing mutations (e.g., E1371S) that abolish ATP hydrolysis. However, in the absence of ATP, R352C/E1371S channels exhibit only C $\leftrightarrow$ O $\leftrightarrow$ C transitions, indicating that without ATP-induced NBD dimerization, the pore conformation must be different from that opened by ATP-induced NBD dimerization. Statistical analysis of single-channel gating events also revealed a considerable amount of opening events containing more than one O1-O2 (i.e. C-(O1-O2)-C) transition. If we accept the idea that the O1-O2 transition represents the hydrolysis of 1 ATP molecule, these surprising gating transitions would reflect openings embedded with hydrolysis of more than one ATP molecule\_a violation of one-to-one stoichiometry. These new results lead us to propose a new gating model that features energetically coupled NBDs and TMDs: both NBDs and TMDs hold a certain degree of autonomy to function on their own but conformational changes in each domain are energetically coupled. Importantly, this new model offers a new target for the action of CFTR potentiators.

### 3218-Pos Board B373

#### Conformational Changes in the Catalytically Inactive Nucleotide Binding Site of CFTR

Csaba Mihályi<sup>1</sup>, Andras Szollosi<sup>1</sup>, Paola Vergani<sup>2</sup>, Laszlo Csanady<sup>1</sup>.

<sup>1</sup>Semmelweis University, Budapest, Hungary, <sup>2</sup>University College London, London, United Kingdom.

The nucleotide binding domains (NBDs) of CFTR can form a dimer with two nucleotide molecules bound at the interface. CFTR channel opening and closing are coupled to formation and partial separation of this NBD dimer. In CFTR the two interfacial binding sites (site 1 and 2) are functionally different. In a regular gating cycle the canonical, catalytically active, nucleotide binding site (site 2) cycles between dimerized prehydrolytic (state O1), dimerized posthydrolytic (state O2), and dissociated (state C) forms in a preferential C $\rightarrow$ O1 $\rightarrow$ O2 $\rightarrow$ C sequence (1). In contrast, the catalytically inactive nucleotide binding site (site 1) was suggested to remain associated, and ATP-bound for several gating cycles (2-3). Here we have examined the possibility of conformational changes in site 1 during gating, by studying gating effects of perturbations in site 1.

Previous work showed that N6-(2-phenylethyl)-ATP (P-ATP) slows both hydrolytic and non-hydrolytic closure by binding to site 1 (2). We found that P-ATP decreases non-hydrolytic closing rate (transition O1 $\rightarrow$ C) of CFTR mutants K1250A (~4x) and E1371S (~2.5x), and prolongs wild-type CFTR burst durations by selectively slowing (>2x) transition O1 $\rightarrow$ O2. Introducing the H1348A (NBD2 signature sequence) mutation into site 1 also decreased non-hydrolytic closing rate of both K1250A (>2x) and E1371S (>2x) CFTR, and in the wild-type background slowed (~3x) the O1 $\rightarrow$ O2 transition. In contrast, the site-1 mutation K464A (NBD1 Walker-A motif) increased non-hydrolytic closing rate (~10x for K1250A, ~10x for E1371S) while decreasing (~5x) rate O1 $\rightarrow$ O2 in a wild-type background.

The fact that P-ATP bound at site 1 as well as several site-1 mutations significantly affect the rates of both steps O1 $\rightarrow$ C and O1 $\rightarrow$ O2 suggests conformational changes in site 1 occur during these steps of the gating cycle.

(1) PNAS 107:1241-1246.

(2) J Gen Physiol. 135:399-414.

(3) J Gen Physiol. 137:549-562.

### 3219-Pos Board B374

#### Pore Blocker Reports Gating-Conformational Changes of the CFTR Channel

László Csanády.

Semmelweis University, Budapest, Hungary.

The CFTR chloride channel is an ABC protein which couples ATP binding and hydrolysis at two conserved intracellular nucleotide binding domains (NBDs) to gating conformational changes. In all ABC proteins ATP binding at both NBDs drives formation of a stable NBD-dimer occluding two ATP molecules at the interface. ATP hydrolysis prompts dimer dissociation, to allow initiation of a new cycle following ADP-ATP exchange. CFTR pore gating is coupled to this irreversible cycle such that dimerized NBDs correspond to an open, while dissociated NBDs to a closed pore (*Nature* 433:876-880): under normal conditions, most openings follow the gating sequence C $\rightarrow$ O1 $\rightarrow$ O2 $\rightarrow$ C, where state

C is a compound closed state, O1 is a prehydrolytic, and O2 a posthydrolytic open state (*PNAS* 107:1241-46). Whereas C $\leftrightarrow$ O transitions involve pore opening/closure, little is known about the conformational changes associated with the hydrolytic O1 $\rightarrow$ O2 step. NPPB is a voltage-dependent pore blocker which also stimulates CFTR open probability (*J. Biol. Chem.* 280:23622-23630). Here we have used macroscopic and single-channel recordings to dissect the mechanism of its gating effect. We found that NPPB prolongs wild-type (WT) CFTR open times by ~4x, due to selective slowing of the O1 $\rightarrow$ O2 step. In addition, NPPB appears to stabilize the C $\leftrightarrow$ O1 transition state, because it increases by ~3x both the opening rate of WT channels, and the closing rate of non-hydrolytic catalytic-site mutants. In contrast, CFTR gating is not affected by the pore blocker MOPS. Gating effects of NPPB are voltage-independent, and cannot be competed off by the presence of MOPS, suggesting that these effects involve NPPB binding to a site outside the pore; this site is likely located in the transmembrane domain, because NPPB gating effects are also insensitive to [ATP] and do not require the presence of the R domain.

### 3220-Pos Board B375

#### Arginine Movement in Synergistic Coupling of Substrate and Ion Binding to Glutamate Transporter Homologue

Secheol Oh, Olga Boudker.

Weill Cornell Medical College, New York, NY, USA.

Glutamate transporters are membrane pumps, which catalyze reuptake of the neurotransmitter from the brain synapses driven primarily by symport of sodium ions. A bacterial homologue, GltPh is a sodium/aspartate symporter, for which the crystal structures of sodium and substrate bound states have been determined. The thermodynamic studies have demonstrated that binding of sodium ions and aspartate to the transporter are coupled, such that the affinity for the substrate is much higher in the presence of the ions and vice versa. However, the ions are not directly coordinated by the substrate and the molecular mechanism of coupling remains largely unknown. Arginine 397 interacts with the side chain carboxylate of the substrate in the transporter crystal structures, is highly conserved among glutamate transporters, and has been shown in functional studies to be a key residue for substrate binding in mammalian homologues. To probe how the formation of the substrate-binding site affects sodium binding, we generated R397A mutant. We show that, as expected, R397A mutant has a low substrate affinity even in the presence of 100 mM sodium, measured by the isothermal titration calorimetry. Remarkably, in the absence of aspartate the sodium binding affinity for R397A is increased by ~10 fold, compared to the wild type transporter, as measured by a fluorescence assay in vitro. Furthermore, substrate does not significantly increase the affinity of the mutant transporter for the ions. We suggest that arginine 397 in the wild type GltPh interferes with the sodium binding site or sites in the absence of the substrate and that its re-orientation upon substrate coordination relieves this interference. We hypothesize that these events are key to the mechanism of the allosteric coupling between substrate and ion binding.

### 3221-Pos Board B376

#### A Water-Organizing Residue for H<sup>+</sup> Access in a CLC Cl<sup>-</sup>/H<sup>+</sup> Antiporter

Hyun-Ho Lim, Tania Shane, Christopher Miller.

Brandeis University / HHMI, Waltham, MA, USA.

Chloride-transporting membrane proteins of the CLC family appear in two distinct mechanistic flavors: H<sup>+</sup>-gated Cl<sup>-</sup> channels and Cl<sup>-</sup>/H<sup>+</sup> antiporters. Transmembrane H<sup>+</sup> movement is an essential feature of both types of CLC. X-ray crystal structures of CLC antiporters show the Cl<sup>-</sup> ion pathway through these proteins, but the H<sup>+</sup> pathway is known only inferentially by two conserved glutamate residues that act as way-stations for H<sup>+</sup> in its path through the protein. The extracellular-facing H<sup>+</sup> transfer glutamate becomes directly exposed to aqueous solution during the transport cycle, but the intracellular glutamate E203, Glu<sub>in</sub>, is buried within the protein. Two regions, denoted "polar" and "interfacial," at the intracellular surface of the bacterial antiporter CLC-ec1 are examined here as possible pathways by which intracellular aqueous protons gain access to Glu<sub>in</sub>. Mutations at multiple residues of the polar region have little effect on antiport rates. In contrast, mutation of E202, a conserved glutamate at the protein-water boundary of the interfacial region, leads to severe slowing of the Cl<sup>-</sup>/H<sup>+</sup> antiport rate. The x-ray crystal structure of E202Y, the most severely affected mutant, suggests that the aromatic ring of Y202 makes cross-subunit hydrophobic interaction with an I201 sidechain from neighboring subunit, which physically blocks the interfacial pathway. This mechanism is functionally supported by the minimal effect of E202Y mutation in a monomeric variant of the transporter, which does not have such an interaction. The several lines of experiments presented argue that E202 acts as a water-organizer that creates a proton conduit connecting intracellular solvent with Glu<sub>in</sub>.